British Microbiology Research Journal
11(6): 1-6, 2016, Article no.BMRJ.22372
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Phytochemical Study and Antibacterial Properties of the Leaf Extracts of *Swartzia madagascariensis* Desv (Fabaceae)

Muhammad Hassan Sani[1*], Garba Ibrahim[1], Umar Habibu Danmalam[1], Zakariya Ali Muhammad[1] and Muhammad Kachallah[2]

[1]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MHS and MK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors MHS, GI, UHD and ZAM managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/22372
Editor(s):
(1) Marcin Lukaszewicz, Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland and Division of Chemistry and Technology Fuels, Wroclaw University of Technology, Wroclaw, Poland.
Reviewers:
(1) Ofentse Mazimba, University of Botswana, Botswana.
(2) Arimah, Babatunde David, Igbinedion University, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/12458

Original Research Article

Received 29th September 2015
Accepted 6th November 2015
Published 27th November 2015

ABSTRACT

This study evaluated the phytochemical and antibacterial properties of the hexane, ethyl acetate and ethanol leaf extracts of *Swartzia madagascariensis* Desv (Fabaceae). Ethno-medicinally, the leaves are used in the treatment of cutaneous wounds, diarrhoea, malnutrition, inflammations and scabies among others. The phytochemical screening using standard methods, revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, saponins, triterpenes and steroids. The antibacterial activities of extracts (2.5, 5, 10, 20 and 40 mg/ml) of *S. madagascariensis* were tested against three Gram-positive—*Staphylococcus aureus, Streptococcus pyogenes,* and *Corynebacterium ulcerans*; one Gram-negative—*Escherichia coli* pathogens. The activity was determined using well diffusion method with zones of inhibition ranges of 17-18 mm for hexane, 24-27 mm for ethyl acetate and 21-22 mm for ethanol extracts. Minimum Inhibitory Concentration (MIC)

**Corresponding author: E-mail: mhsloko26@gmail.com;*

of the extracts was determined using 0.5 scale Mc-farland's turbidity standard. The extracts at 20 mg/ml (hexane), 5-10 mg/ml (ethyl acetate) and10 mg/ml (ethanol) inhibited the growth of the isolates. It also gives Minimum Bactericidal Concentrations ranging from 40 mg/ml (hexane extract), 20 mg/ml (ethyl acetate) and 40 mg/ml (ethanol extract). Zone of inhibitions of extracts were compared to that of standard antibacterial drug, ciprofloxacin (32-37 mm). The results from this study support the traditional use of the leaves of *S. madagascariensis* in the treatment of bacterial infections.

Keywords: Swartzia madagascariensis; leaf extracts; phytochemical screening; antibacterial activities.

1. INTRODUCTION

Infectious diseases are one of the leading causes of morbidity and mortality worldwide, especially in developing countries [1]. Therefore, the use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial agents with significant activity against infective bacterial strains [2].

Infections caused by resistant bacteria often fail to respond to the standard treatment, resulting in prolonged illness and greater risk of death. The death rate for patients with serious infections treated in hospitals is about twice that in patients with infections caused by non-resistant bacteria [3]. Alternative antibacterial drugs from plants have been revived for disease management due to the increased prevalence of multidrug resistance strains of bacterial isolates. This increased prevalence have been attributed to the indiscriminate uses of commercial antibiotics and this in turn has forced scientist to search for new antibacterial substances from various medicinal plants. These plants appears to be the important approaches to the development of these antibiotics as metabolites such as phenolic compounds and essentials oils have been reported to posses high antibacterial properties which are of great importance to tackling bacterial diseases [4].

Swartzia madagascariensis Desv is a member of the plant family *Fabaceae*. It is a semi deciduous shrub or small tree up to 15 m tall, multi-stemmed or with a single bole up to 60 cm in diameter. The leaves are compound, imparipinnate with a common stalk of 7.5-10 cm long with short hairs. It has 5-11 alternate to sub-opposite leaflets, and the leaflets are elliptic to obovate. Fruits are cylindrical with dark brown to black colour, up to 30 cm, indehiscent. Flowers have 2-10 flowered sprays, with one large petal and a mass of orange yellow stamens, sweetly scented [5]. It is called Snake bean in English, 'Bayama' or 'Gama Fada' in Hausa, 'Yawolawogi' in Nupe, 'Hil Igwom' in Tiv. *Swartzia madagascariensis* leaves have been used traditionally in northern Nigeria as a remedy against some infectious diseases like cutaneous wounds, scabies and venereal diseases [6]. The use of herbal drugs in traditional medicine needs to be evaluated by using current scientific approaches with the view to giving the patient an appropriate dosage of the medication as against the most practiced unquantifiable approach by the native healers [1].

The present study is aimed at investigating the phytochemical constituents and antibacterial activities of the leaf extracts of *S. madagascariensis* with a view to either support or debunks the traditional claims of using the leaf in treatment of bacterial infections.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Preparation

Fresh leaf of *S. madagascariensis* was collected around Kufena village in Zaria Local Government area, Kaduna State, Nigeria in June, 2014. It was identified at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria Nigeria. The plant was identical to a herbarium sample with voucher number 7231 deposited in the Department. The plant sample was dried for five days at room temperature, powdered, sieved and stored at a room temperature, in a closed container for future use.

2.2 Extraction of Plant Materials

The plant material (500 g) was extracted with two liters of Hexane using soxhlet apparatus and the extract was filtered and marc was pressed to dryness and then further extracted with two liters of ethyl acetate and finally with ethanol and the extracts were then filtered using a Whatmans

No. 1 filter paper and the extracts were evaporated to dryness using a water bath at 40℃. The dried extracts were weighed and the percentage yield for the plant extracts were hexane (1.90%w/w), ethyl acetate (10.73%w/w) and ethanol (5.34%w/w) respectively and kept in a desiccator until required for further analysis.

2.3 Phytochemical Screening of the Crude Extracts of *S. madagascariensis* Leaf

Phytochemical analysis was carried out to determine the active ingredients of the hexane, ethyl acetate and ethanol extracts of the *S. madagascariensis* leaves. Procedures described by [7] were adopted for the detection of the presence or absence of alkaloids, anthracenes, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes.

2.4 Antibacterial Activity

2.4.1 The test microorganisms

The test organisms were biochemically identified clinical isolates of *Corynebacterium ulcerans, Escherichia coli, Staphylococcus aureus and Streptococcus pyogenes.* They were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika.

2.4.2 Standardization of the inoculum

The bacterial isolates were sub-cultured in nutrient broth for 24 hours. A loopful of the overnight nutrient broth was diluted in normal saline (0.85% NaCl w/v). The turbidity marched with 0.5 Mcfarland standards, which contained a mean of $1.5x10^8$ CFU/mL, which marches with the standard turbidity of 1% (w/v) barium sulphate solution [8].

2.4.3 Preparation of extract concentrations

Hexane, ethyl acetate and ethanol leaf extracts (0.4 g) each of *S. madagascariensis* were weighed and dissolved separately in 10 ml of DMSO to obtain a concentration of 40 mg/ml as an initial concentration. From the stock solution, two-fold serial dilutions are made to obtain 20, 10, 5 and 2.5 mg/ml concentration of each extracts. Standard antibiotic – Ciprofloxacin (10 µg) was used as the positive control drug for antibacterial agents [9].

2.4.4 Preparation of culture media

Culture media for antibacterial study was prepared using the Muller Hinton agar and was sterilized at 121℃ for 15 minutes, poured into sterilized petri dishes; it was then allowed to cool and solidified. Diffusion method was used to screen the extracts; the sterilized medium was seeded with 0.1 ml of the standard inoculums of the test microbes and was spread evenly over the surface of the medium with a sterile swab. A well was cut at the centre of each inoculated medium using a standard cork – borer of 6 mm in diameters; 0.1 ml of the extract solution of the concentration of 40 mg/ml was introduced into each well on the inoculated medium.

The incubation of the medium was made at 37℃ for 24 hours, after which the plates were observed for the zone of inhibition of growth which was measured with a transparent ruler and the result recorded in millimeters [9,10].

2.4.5 Determination of MIC

The minimum inhibitory concentrations of the different extracts were carried out using the broth dilution method [11].

Mueller Hinton broth was prepared, 10 ml was dispensed into test tubes and sterilized at 121℃ for 15 minutes and the broth was allowed to cool. The turbidity was made possible by preparing the Mc – farland's turbidity scale number 0.5. The test microbes were inoculated into the test tubes by dissolving 10 ml of the prepared normal saline and then incubated at 37℃ for 6 hours. Dilution of the test microbes was done in the normal saline until the turbidity marched with that of the Mc – farland standard by visual comparison and the concentration was determined at that point. Two – fold serial dilution of the extract in the sterile broth was prepared and concentrations were obtained as 40, 20, 10, 5 and 2.5 mg/ml.

The test microbes (0.1 ml) in the normal saline were inoculated at different concentrations, incubated at 37℃ for 24 hours after which the test tubes are observed for turbidity (growth). The lowest concentration of the extract in the broth that will not allow turbidity indicates minimum inhibitory concentration [11].

2.4.6 Determination of MBC

Minimum bactericidal concentrations of the different extracts were carried out to determine whether the test microbes were killed or their growth was inhibited. The contents of the MIC in the serial dilution was then sub – cultured onto the prepared medium and incubated at 37℃ for 24 hours, after which each plate of the medium was observed for colony growth. The minimum bactericidal concentration was determined as the plates with lowest concentration of the extract without colony growth [11].

3. RESULTS AND DISCUSSION

Table 1 presents the phytochemical constituents of the hexane, ethyl acetate and ethanol extracts of *S. madagascariensis* leaf. Alkaloids, anthracenes, cardiac glycosides, flavonoids, saponins, steroids, tannins and steroids were found to be present in the ethanol extract, while flavonoids, steroids and triterpenes were present in the ethyl acetate extract. The hexane extract contained chiefly steroids and terpenoids, while alkaloids, anthracenes, flavonoids and tannins were not detected. These results are almost in conformity with [12].

Table 2 shows the antibacterial activities of the hexane, ethyl acetate and ethanol extracts of *S. madagascariensis* leaf. Ciprofloxacin (10 µg) used as a control was found to be active against all the test bacterial isolates with diameter of inhibition zones ranging between 32 mm to 37 mm. Hexane extract was found to be inactive against *C. ulcerans,* but was active against the rest of the test bacteria with diameter of zones of inhibition ranging between 17 mm to 18 mm. Ethyl acetate extract exhibited strong activity against gram positive*, S. aureus* at a diameter zone of inhibition of 27 mm and *S. pyogenes* with diameter zone of inhibition of 25 mm; while it was in- active against *C. ulcerans*, and for the gram negative bacteria was recorded as *E. Coli* at 24 mm. The ethanol extract also has an effect on the bacterial isolates with diameter zones of inhibition of 22 mm for *S. aureus*, 21 mm for *S. pyogenes,* while it has no activity on *C. ulcerans* and 22 mm for *E. coli*. The hexane extract had minimal effect on the test organisms compared to ethyl acetate and ethanol extracts, with diameter zones of inhibition of 18 mm for *S. aureus,* 18 mm for *S. pyogenes*, no effect against *C. ulcerans* and 17 mm for *E. coli*.

Table 3 presents the results of MIC of the hexane, ethyl acetate and ethanol extracts of *S. madagascariensis* leaf. The MIC was 10 mg/ml for the ethyl acetate extract against all pathogens except for *S. aureus* which was observed at 5 mg/ml, MIC of the ethanol extract was observed at 10 mg/ml against all pathogens. Whereas, MIC of the hexane extract was at 20 mg/ml. Ethyl acetate extract had exhibited better activity at MIC of 5 mg/ml compared to ethanol extract with MIC of 10 mg/ml and hexane extract with MIC of 20 mg/ml.

Table 4 shows the results of MBC of the hexane, ethyl acetate and ethanol extracts of *S. madagascariensis* leaf. Ethyl acetate had a better activity against all the test isolates at MBC of 20 mg/ml followed by ethanol that was active at MBC of 40 mg/ml against the bacterial strains. Hexane extract was active at MBC of 40 mg/ml against all isolates (*E. coli, Staph. aureus* and *S. pyogenes*).

The presences of phenolic compounds which are known to have antibacterial activity were revealed in the plant; this therefore, supported the use of the plant in the traditional treatment of cutaneous infections, venereal diseases, and dysentery [6].

Table 1. Phytochemical screening of the leaf extracts of *S. madagascariensis*

Phytoconstituents	Hexane	Ethyl acetate	Ethanol
Alkaloids	-	-	+
Anthracenes	-	-	+
Cardiac glycosides	-	-	+
Flavonoids	-	+	+
Saponins	-	-	+
Steroids	+	+	+
Tannins	-	-	+
Triterpenes	+	+	+

Key: + = Present, - = Absent

Table 2. Zones of Inhibition of hexane, ethyl acetate and ethanol extracts (mm) of *S. madagascariensis* leaf

Test organisms	Hexane	Ethyl acetate	Ethanol	Ciprofloxacin
Corynebacterium ulcerans	0	0	0	32
Escherichia coli	17	24	22	35
Staphylococcus aureus	18	27	22	37
Streptococcus pyogenes	18	25	21	35

Table 3. Minimum Inhibitory concentration of hexane, ethylacetate and ethanol extracts of *S. madagascariensis* leaf

Organisms	Hexane (mg/ml)					Ethyl acetate (mg/ml)					Ethanol (mg/ml)				
	40	20	10	5	2.5	40	20	10	5	2.5	40	20	10	5	2.5
C. ulcerans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. coli	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++
Staph. aureus	-	*	+	++	+++	-	-	-	*	+	-	-	*	+	++
Strep. pyogenes	-	*	+	++	+++	-	-	*	+	++	-	-	*	+	++

*KEY: - = clear (no growth), * = MIC, + =turbid (light growth), ++ = (moderate turbid), +++ = (high turbidity), 0 = no activity*

Table 4. Minimum bactericidal concentration of hexane, ethyl acetate and ethanol extracts of *S. madagascariensis* leaf

Organisms	Hexane (mg/ml)					Ethyl acetate (mg/ml)					Ethanol (mg/ml)				
	40	20	10	5	2.5	40	20	10	5	2.5	40	20	10	5	2.5
C. ulcerans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E. coli	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
Staph. aureus	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++
S. pyogenes	*	+	++	+++	+++	-	*	+	++	+++	*	+	++	+++	+++

*KEY: - = clear (no growth), * = (MBC), + = scanty colonies growth (light growth), ++ = (moderate turbid colonies growth), +++ = (heavy colonies growth), 0 = no activity*

Michal et al. [13] reported that saponins are sensitive against six strains of *E. coli* compared to standard drugs streptomycin. Active crude flavonoids separated from *Mimba pudica* (Fabaceae*)* have been evaluated and found sensitive against Gram positive *Staph. aureus* and gram negative *P. aeruginosa* [14].

4. CONCLUSION

The results of the phytochemical screening showed that alkaloids, anthracenes, cardiac glycosides, flavonoids, saponins, steroids, tannins and triterpenes were present in varied compositions in the leaf extracts (hexane, ethyl acetate and ethanol) of *S. madagascariensis*.

The results of the sensitivity test also showed that all the leaf extracts of *S. madagascariensis* had antibacterial activities against the tested bacterial isolates. Also, values of MBC and MIC indicate that the ethyl acetate extract had the most bactericidal properties on the studied isolates. This activity could be attributed to flavonoids, steroids or triterpenes.

5. RECOMMENDATION

Further work needs to be done to determine, identify, purify and quantify the antibacterial compound within this plant and also to determine its full spectrum of efficacy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. The World Health Organisation Report Life in 21st Century: A vision for all to measure health. WHO, Geneva, Switzerland. 1998;39- 60.

2. World Health Organization. Guidelines on Good Agricultural and Collection Practices (GACP) for medicinal plants; 2003. Available:http:/whqlibdoc.who.int/publications/2003/9241546271 (Accessed on 3 September, 2009)
3. WHO. Antimicrobial Resistance, 2014; Fact sheet No 194 updated May, 2014. Geneva, Switzerland.
4. Gowsiya S, Sujaya N, Santosh KM. Medicinal plants as source of antibacterial agents to counter *Klebshiella pneumoniae*. Journal of Applied Pharmaceutical Sciences. 2014;4(01):135-147.
5. Amri E. The effect of auxins (IBA, NAA) on vegetative propagation of medicinal plant *Bobgunnia madagascariensis* (Desv.) J.H. Kirkbr & Wiersema. Tanzania Journal of Natural and Applied Sciences. 2011;2(2): 359–366.
6. Tom EG, Silje G, Mona S, Ngolo B, Sekou, B, Drissa D, Berrit SP. Ethnopharmacological survey of six medicinal plants from mali, West Africa. Journal of Ethnobiology and Ethnomedicine. 2008;4:26.
7. Sofowora A. Medicinal plants and traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria. 3rd Edition. 2008;200-202.
8. Kawo AH, Kwa AM. Phytochemical screening and antibacterial activity of the aqueous extracts and fractions of ethanolic extracts of *Lawsonia inermis* Leaf. International Research Journal of Microbiology. 2011;2(12):510-516.
9. Azoro C. Antibacterial activity of crude extracts of *Azadirachta indica* on *Salmonella typhi.* World Journal of Biotechnology. 2002;3:347-357.
10. Mobasher M, Sahraei H, Sadeghi RB, Kamalinejad M, Shams J. Rafsanjan Journal of Medicine. 2005;5:143-150.
11. EUCAST Definitive Document. Terminology relating to the methods for the determination of susceptibility of bacterial to antimicrobial agents. Clinical Microbiology Infection. 2000;6(9):503-509.
12. Neuwinger HD. Plants used for poison fishing in Tropical Africa. Toxicon. 2004; 44(4):417- 30.
13. Michal A, Aneta WC, Grzegorz C, Anna L, Wieslaw K. Effect of saponin against clinical *E. coli* strains eukaryotic cell lines. Journal of Biomedicine and Biotechnology. 2012;12:6. DOI:http://dx.coi.org/10.1155/2012/286216
14. Doss A, Vijayasanthi M, Arivuguna VP, Anand SP. Evaluation of antibacterial properties of ethanol and flavonoids from *Mimosa pudica* Linn and *Panicum maximum* Jacq. Journal of Plant Sciences Feed. 2011;1(2):39-44. Available:http://pgf.lifescifeed.com

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12458